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EFFECT OF LEAD ON THE ACTIVITY OF SOME ENZYMES OF NITROGEN METABOLISM IN SUGAR BEET *(BETA VULGARIS* L.)

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The subject of the study, a solution culture pot experiment, was the effect of lead on nitrate accumulation, lead content, the activity of some enzymes of nitrogen metabolism (nitrate reductase, glutamine synthetase, and glutamate dehydrogenase), the content of chloroplast pigments and the dry matter mass of young sugar beet plants *(Beta vulgaris* L., hybrid NS-Hy 11). The plants were treated with 10^{-5} or 10^{-3} M lead solutions. The lead concentrations inhibited NR, GS and GDH activity and reduced chloroplast pigments content, but not the nitrate concentration in the leaves. Lead content significantly increased with an increase in lead concentration in the nutrient solution, especially in the root, while the dry matter mass of the above-ground plant parts and the root decreased.

Key words: *Beta vulgaris* L., lead concentration, nitrates, nitrate reductase, glutamine synthetase, glutamate dehydrogenase, chloroplast pigments, dry matter

Ključne reči: *Beta vulgaris* L., koncentracija olova, nitrati, nitrat-reduktaza, glutamin-sintetaza, glutamat-dehidrogenaza, pigmenti hloroplasta, suva masa

INTRODUCTION

The availability of heavy metals in the soil depends on natural processes, especially on lithogenic and pedogenic ones, but also on anthropogenic factors (Filipinski and Grupe, 1990).

Anthropogenic factors, such as industrial activity and mining (Woolhouse, 1983), sewage disposal (Zurera-Cosano and Moreno-Royas, 1990), traffic (Sommer and Stritesky, 1976) etc, are the main factors responsible for an increase in the concentration of heavy metals in the soil.

Heavy metal-induced stress causes various direct and indirect effects on practically all physiological processes in plants (Woolhouse, 1983). The primary toxicity mechanisms of heavy metals alter the catalytic function of enzymes (Van Assche and Clijsters, 1990; Petrović et al., 1990), damage cellular membranes (Tu and Brouillette, 1987) and inhibit plant growth (Láng et al., 1995). These changes cause numerous secondary effects such as inhibition of photosynthesis (Lang et al., 1995) and mineral nutrient uptake (Nunes et al., 1995), hormonal imbalance and water stress (Barcelo et al., 1986; Kastori et al., 1993), different structural and ultrastructural changes (Vasquez et al., 1987), etc.

A considerable number of authors think that heavy metals primarily inhibit enzyme activity and/or cause structural changes in proteins, since they have the ability to interact with proteins, especially structural ones, through their sensitive SH- or histidil groups (Rauser, 1993). Having in mind that the enzymes of nitrogen assimilation contain amino acids rich in sulfur, we thought it would be worthwhile to investigate the effect of lead on the activities of some enzymes of nitrogen metabolism.

MATERIAL AND METHODS

Experiments with young sugar beet plants *(Beta vulgaris* L., hybrid NS-Hy 11) were conducted in semi-controlled greenhouse conditions. Having been allowed to germinate in the vermiculite, the seedlings were transferred into solution-culture pots, on 1/2 Hoangland's solution, which has the following composition [mM]: 2.5 $Ca(NO_3)_2$; 2.5 KNO_3; 1.0 KH_2PO_4; 1.0 $MgSO_4 \cdot 7H_2O$ and [μM]: 21.3 B; 4.6 Mn; 0.38 Zn; 0.052 Mo; 0.15 Cu and 8.95 Fe as $Fe_{(III)}$NaEDTA. Three weeks after this, the plants were grown alternatively on 1/2 Hoangland's solution and in the presence or 10^{-5} of 10^{-3}M Pb, each turn lasting 24 h. The plants were grown in this manner for twelve days, after which symptoms of the excess of lead became clearly visible. They were then picked and the above-ground plant parts and roots separated from one another. The dry matter mass of each organ was determined after drying the samples at 70°C to constant weight. Nitrate reductase (NR) activity was determined *in vivo,* in phosphate buffer (pH 7.4) (Hageman and Reed, 1980). The activity of the *in vitro* transferase reaction of glutamine synthetase (GS) and NADH-dependant glutamate dehydrogenase (GDH) was determined in a common leaf extract according to Coombs and Hall (1982). The lead concentration was established using atomic absorption spectrophotometry. The nitrate content was determined by spectrophotometric assay with phenoldisulfic acid. The levels of chlorophyll *a, b* and carotenoide content were determined spectrophotometrically in the acetone extract of freshly harvested leaves, using molar extinction coefficients according to Holm (1954) and von Wettstein (1957).

The results were statistically processed by calculating the lowest statistically significant differences (LSD).

RESULTS AND DISCUSSION

The dry matter mass of the above-ground plant parts and roots decreased with the increase in lead concentration in the nutrient solution (Fig. 1). The mass of the above-ground plant parts was more negatively affected by lead than the root mass. This was indicated by the ratio of the dry matter mass of the above-ground plant parts and the dry matter mass of the root in the presence of the lead concentrations studied. This ratio was 4.44 in the check tretment, and 3.79 in plants grown in the presence of higher concentrations of lead (10^{-3}M). This suggests that the above-ground plant parts of sugar been are much more sensitive to the presence of higher lead concentrations then the sugar beet root, which is confirmed by the concentration of lead in the above-ground plant parts and in the root. The concentration was much higher in the root, the mass of which decreased to a smaller extent, than in the above-ground plant parts (Fig. 2). Therefore, the deposition of Pb in the root may be considered a form of self-detoxification on the part of plants. Different compounds and ions are involved in the precipitation of Pb within plants, which is thus rendered metabolically inactive (K n e e r and Z e n k , 1992).

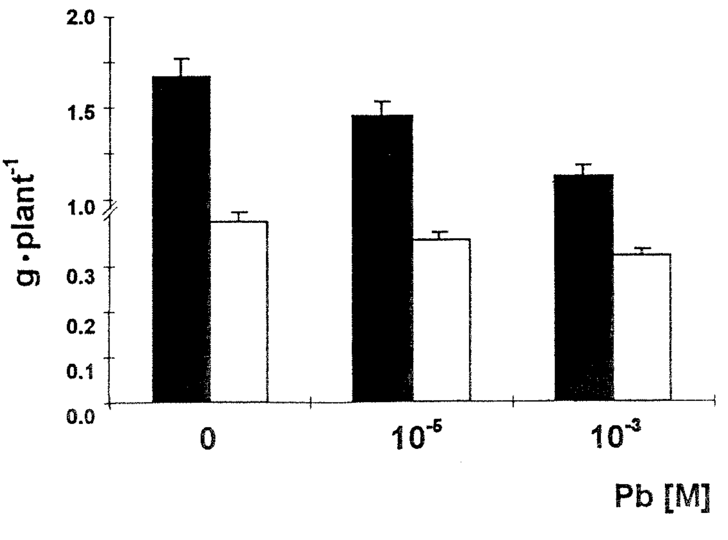

Fig. 1. – The effect of different lead concentrations on dry matter mass of the above-ground plant parts (■) and roots (□) of young sugar beet plants.

The level of chlorophylls *a* and *b* and carotenoids decreased in the presence of lead in the nutrient solution, whereas the chlorophyll *a*: chlorophyll *b* ratio increased (Fig. 3). In maize (G a š i ć et al., 1992) and cucumber (L á n g et al., 1995) Pb displayed similar effect on chloroplast pigments content. A significant number of authors are of the opinion that heavy metals inhibit chlorophyll biosynthesis by causing a decrease in

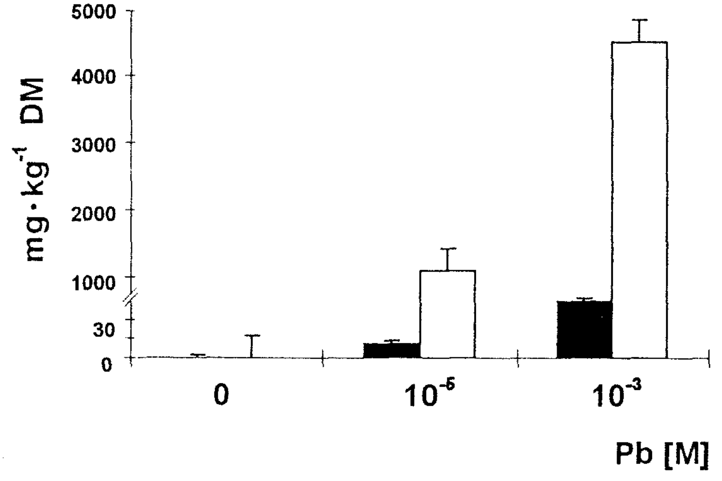

Fig. 2. – The effect of different lead concentrations on lead content in the above-ground plant parts (■) and roots (□) of young sugar beet plants

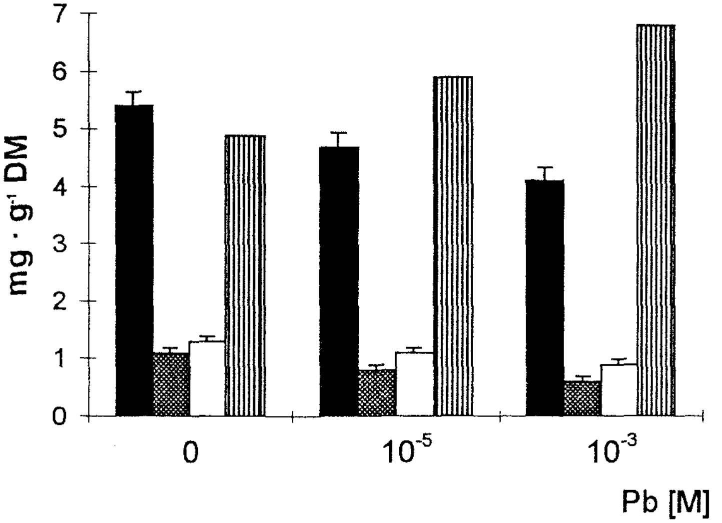

Fig. 3. – The effect of different lead concentrations on the content of chloroplast pigments in young sugar beet plants: chlorophyll *a* (■) chlorophyll *b* (▩), carotenoids (□) and chlorophyll *a*: chlorophyll *b* ratio (▥)

the sum of reducing equivalents and ATP synthesis. This way, altering the activity of RUBP-carboxylase, they indirectly effect the uptake and metabolism of CO_2 (Láng et al., 1995).

The nitrate content in the leaves of sugar beet significantly increased with an increase in lead concentration in the nutrient solution (Fig. 4). Nitrate content may increase not only as a result of an excessive application of nitrates – it can also be caused by numerous internal and external factors, such as environmental pollution with heavy metals, which may also act as indirect inhibitors of photosynthesis and respiration (Vale and Ulmer, 1972). Under such conditions the energy pool and the sum of reductive equivalents necessary for nitrate assimilation are reduced. On a number of occasions it has been shown that the majority of non-essential heavy metals inhibit the activity of nitrate reductase, the key enzyme in the process of nitrate reduction (Burzynski and Grabowski, 1984; Petrović et al., 1990; Kastori et al., 1996). Besides, it is likely that Pb activates the transfer of one part of the nitrate from the „metabolic pool" into the „reserve pool" (Oaks et al., 1989).

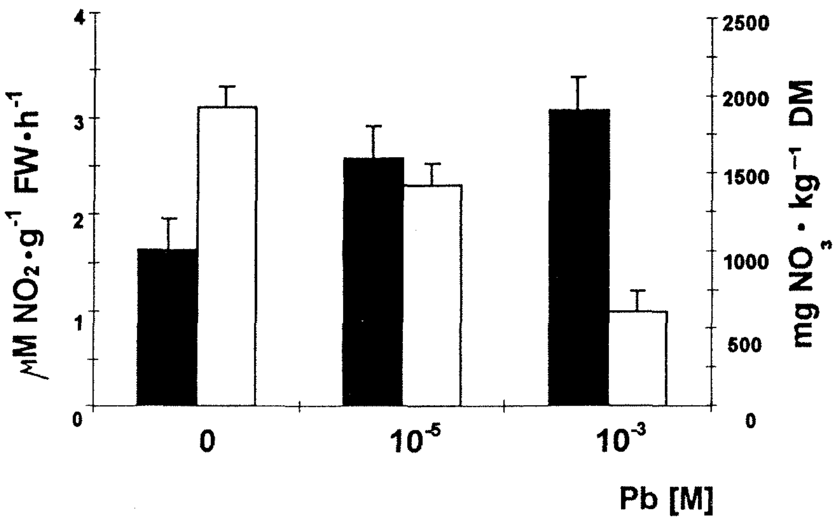

Fig. 4. – The effect of lead on NRA (□) and nitrate content (■) in the above-ground parts of young sugar beet plants

NR activity in the leaves decreased with an increase in Pb concentration (Tab. 4). Similar results were obtained for *Pisum sativum* and *Helianthus annuus*, indicating that Pb has a direct effect on enzyme synthesis (Sinha et al., 1988). According to Burzynski and Grabowski (1984), lower lead concentrations in the nutrient solution affect NR activity indirectly, probably through water stress, whereas the high concentrations directly affect the proteins that build the enzyme structure.

A study of the Pb effect on the activity of nitrogen assimilation enzymes in the leaves of *Pisum sativum* L. showed that NR was most sensitive to the presence of this metal (Sinha et al., 1988).

GDH activity in leaves was inhibited at both of the Pb concentrations applied, thus inhibiting the reductive amination of α-ketoglutarate as well (Fig. 5). These results are in agreement with previous observations regarding Pb-induced GDH inhibition in maize (Kastori et al., 1993) and Cd-induced inhibition in *Phaseolus vulgaris* (Van Assche and Clijsters, 1990).

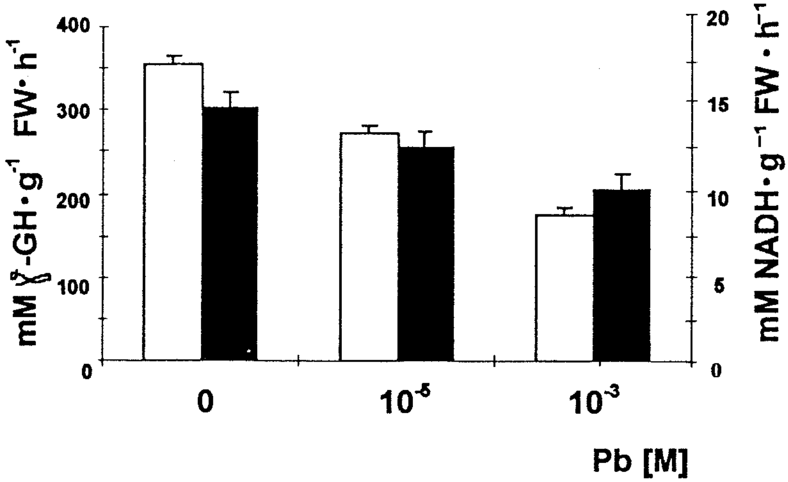

Fig. 5. – The effect of lead on GS (■) and GDH (□) activity in the above-ground part of young sugar beet plants

The presence of Pb had quite similar inhibitory effects on GS activity as well (Fig. 5). Results obtained with lead are similar to those obtained with Cd, which was also shown to inhibit GS activity in sugar beet (Popović et al., 1996).

In the majority of crops GS is present in two isozyme forms: GS_1 and GS_2. It was suggested that isozyme GS_2, which is present in chloroplasts, plays a role in the primary ammonia assimilation and that its activity primarily depends on the amount of light absorbed and the intensity of ATP synthesis (Mc Nally et al., 1983). With respect to this, it can be assumed that the inhibition of GS activity in the presence of lead is likely a results of decreased ATP synthesis (Láng et al., 1995). According to the results of our study, it can be concluded that, either directly or indirectly, lead strongly affects primary nitrogen assimilation. At the same time, the possibility that lead also affects the uptake of nitrogen ions can not be excluded, either (Burzynsky and Grabowski, 1984).

CONCLUSIONS

An increase of the lead concentration in the nutrient solution especially increased the lead content in the leaves, whereas the dry matter mass of both the above-ground plant parts and the root decreased.

The level of chloroplast pigments (chlorophylles *a* and *b* and carotenoids) decreased, but the chlorophyll *a*: chlorophyll *b* ratio was augmented following an increase in lead concentration in the nutrient solution.

The activity of nitrate reductase, glutamine synthetase and glutamate dehydrogenase in lead-treated plants significantly decreased, while the nitrate content significantly increased.

As the enzymes of nitrogen assimilation contain amino acids rich in sulfur, our results support hypothesis after which, in the first place, heavy metals effect functioning of SH-groups and S-S bonds of enzymes.

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Rezime

RUDOLF KASTORI, NOVICA PETROVIĆ, IVANA ARSENIJEVIĆ MAKSIMOVIĆ

UTICAJ OLOVA NA AKTIVNOST ENZIMA METABOLIZMA AZOTA U ŠEĆERNOJ REPI (*BETA VULGARIS* L.)

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Ogledi su izvedeni u polukontrolisanim uslovima, u staklari, na mladim biljkama šećerne repe (hibrid NS-Hy 11). Nakon naklijavanja u vermikulitu, ponici su preneti u posude za vodene kulture i gajene tri nedelje na 1/2 Hoangland-ovom hranljivom rastvoru. Biljke su nakon toga naizmenično (po 24 sata) gajene na Hoangland-ovom hranljivom rastvoru i u prisustvu 10^{-5} ili 10^{-3}M Pb u toku 12 dana, odnosno do pojave jasno vidljivih simptoma suviška olova.

Proučavano je dejstvo različitih koncentracija olova na aktivnost enzima metabolizma azota: nitrat-reduktaze, glutamin-sintetaze i glutamat-dehidrogenaze u nadzemnim delovima biljaka. Pored toga, ispitivano je dejstvo olova na sadržaj olova, nitrata i pigmenata hloroplasta, kao i na masu suve materije nadzemnog dela i korena.

Na osnovu dobivenih rezultata može se zaključiti da se pri povećanju koncentracije olova u hranljivom rastvoru njegova koncentracija naročito značajno povećala u korenu, dok se masa nadzemnog dela i korena smanjila.

Sadržaj pigmenata hloroplasta (hlorofila *a*, *b* i karotenoida) se smanjio, a odnos hlorofil *a* : hlorofil *b* povećao sa povećanjem koncentracije olova u rastvoru.

Aktivnost nitrat-reduktaze, glutamin-sintetaze i glutamat-dehidrogenaze se značajno smanjila, a sadržaj nitrata značajno povećao u biljkama tretiranim olovom.

Imajući u vidu da enzimi asimilacije azota sadrže aminokiseline bogate sumporom, dobiveni rezultati potvrđuju pretpostavku da teški metali prvenstveno utiču na funkcionisanje SH- grupa i S-S veza u enzimima.